



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND CHARACTERIZATION OF MYB-RELATED GENES
FROM OIL PALM (*ELAEIS GUINEENSIS* JACQ.)**

TEOH WAN CHIN

FSMB 2002 27

**ISOLATION AND CHARACTERIZATION OF *MYB*-RELATED GENES
FROM OIL PALM (*ELAEIS GUINEENSIS* JACQ.)**

**By
TEOH WAN CHIN**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

October 2002



Abstract of thesis presented to the Senate of University Putra Malaysia in
fulfillment of the requirements for the degree of Master of Science

**ISOLATION AND CHARACTERIZATION OF *MYB*-RELATED GENES
FROM OIL PALM (*ELAEIS GUINEENSIS* JACQ.)**

By

TEOH WAN CHIN

October 2002

Chairman : Tan Siang Hee, Ph.D.

Faculty : Food Science and Biotechnology

Myb is a sequence specific DNA-binding protein that can activate or inactivate promoters containing its binding site. Plant Myb proteins represent a group of transcription factors which have a DNA-binding domain similar to that found in the products of the animal *myb* proto-oncogenes. Some of the functions, which have so far been assigned to plant *myb* genes, include regulation of phenylpropanoid biosynthesis, control of cellular differentiation, and contribution to control of plant responses to hormones and various stresses.

Two different *myb*-like genes were isolated. *OpMyb24*, which contains a full-length coding region, was obtained from an oil palm zygotic embryo (OPZE) cDNA library. Its Myb-domain shows extensive homology to the snapdragon proteins Myb308 and Myb330, and maize protein Zm38, and these *myb*-like genes have been found to be involved in regulating phenylpropanoid and lignin biosynthesis, and control of flavonoid biosynthesis. The second *myb*-like gene was

isolated from oil palm suspension culture (OPSC) cDNA library, *OpMyb15*, which lacks the 5' end, has greater similarity to MIXTA of *Antirrhinum*, which is essential for the development of the conical form of petal epidermal cells. These two predicted gene products showed high similarity within the myb domain with other *Myb* genes from other species; however, outside of this region virtually no similarity was found.

Expression of *OpMyb24* and *OpMyb15* was detected in all tissues tested, except *OpMyb24* transcript, which was not detected in three-month-old and young leaves. Both *OpMybs* were relatively more abundant in 28-cm male flowers and meristems, which showed distinct, tissue-specific expression patterns.

For the expression study in treated oil palm *in vitro* seedlings, *OpMyb15* and *OpMyb24* transcripts accumulated to high levels in response to gibberellic acids, while the levels of *OpMyb24* decreased significantly after wounding or heavy metals treatment. At the same time, *OpMyb24* mRNA increased slightly after UV light exposure. Much remains to be learned about the function of these oil palm *Myb* genes in their molecular responses in GA-regulated processes, wounding, heavy metal treatment and UV light exposure.

From the Southern blot analyses, these two *OpMybs* were determined to exist as a small gene family. As overexpression or downregulation of a gene is most frequently achieved by the production of transgenic plants carrying sense or antisense copy of the gene, the coding sequence of *OpMyb24* was successfully inserted in both sense and antisense orientations between the 35S CaMV promoter

and *nos* terminator of the expression vector p35S/NOS. The both constructs were consequently subcloned into a binary vector (pCAMBIA 1301) to be used to transform oil palm in the future. This is one of the approaches may provide important clues about the function of *OpMyb24* by studying the phenotype of the resulting transgenic oil palm.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGASINGAN DAN PENCIRIAN GEN-GEN BERHUBUNGAN DENGAN
MYB DARIPADA KELAPA SAWIT (*ELAEIS GUINEENSIS* JACQ.)**

Oleh

TEOH WAN CHIN

Oktober 2002

Pengerusi : Tan Siang Hee, Ph.D.

Fakulti : Sains Makanan dan Bioteknologi

Myb adalah sejenis protin pengikat DNA berjujukan spesifik yang dapat mengaktif atau menyahaktifkan promotor-promotornya. Protin Myb tumbuhan mewakili sekumpulan faktor transkripsi di mana kawasan pengikat DNA berjujukan spesifik mereka mempunyai persamaan terhadap produk *myb* “proto-oncogenes” haiwan. Beberapa fungsi yang selama ini ditugaskan kepada gen *myb* tumbuhan adalah termasuk pengaturan biosintesis “phenylpropanoid”, mengawal pembezaan sel, dan mengawal tindak balas tumbuhan terhadap hormon dan pelbagai tekanan.

Dua gen berlainan yang mempunyai persamaan *myb* telah diasingkan. *OpMyb24* yang mengandungi kawasan pengkodan lengkap diperolehi daripada koleksi “cDNA” embrio zigotik kelapa sawit. Protin “snapdragon” Myb308 dan Myb330, serta protin jagung Zm38 yang terlibat dalam pengawalan biosintesis “phenylpropanoid” dan lignin, serta mengawal biosintesis “flavonoid” mempunyai

tahap persamaan yang tinggi terhadap lingkungan jujukan DNA *Myb* bagi *OpMyb24*. *OpMyb15* adalah gen kedua yang mempunyai persamaan *Myb* diasingkan daripada koleksi “cDNA” kultur ampaian kelapa sawit. Gen ini mengandungi kawasan pengkodan tidak lengkap iaitu kekurangan penghujung 5’, dan mempunyai tahap persamaan tinggi terhadap MIXTA daripada *Antirrhinum*, di mana ia adalah penting dalam pertumbuhan konikal bagi sel ranggi. Kedua-dua jenis gen ini mengandungi tahap persamaan yang tinggi dalam lingkungan jujukan DNA *Myb* dengan gen *Myb* daripada spesis yang lain tetapi tiada persamaan dijumpai langsung di luar lingkungan jujukan DNA *Myb* ini.

Ekspresi *OpMyb24* dan *OpMyb15* dikesan pada semua tisu yang diuji kecuali transkrip *OpMyb24* tidak dapat dikesan pada daun berumur tiga bulan dan daun muda. Kedua-dua *OpMybs* didapati berlebihan di dalam bunga jantan berukuran 28-cm dan “meristem”. Mereka telah menunjukkan corak ekspresi yang jelas terhadap tisu yang spesifik sahaja.

Dalam kajian “*in vitro* seedling”, kelapa sawit yang telah dirawat didapati bahawa transkrip *OpMyb15* dan *OpMyb24* terkumpul banyak sebagai suatu tindak balas terhadap asid gibberelik, manakala paras mRNA *OpMyb24* menurun selepas perlukaan dan rawatan “HgCl₂”. Pada masa yang sama, paras mRNA *OpMyb24* meningkat sedikit selepas terdedah kepada UV. Namun demikian, kajian lanjut diperlukan jika ingin lebih memahami fungsi *Myb* yang diasingkan daripada kelapa sawit terhadap tindak balas molekul dalam proses pengawalaturan GA₃, perlukaan, rawatan HgCl₂ dan pendedahan UV.

Daripada analisis “Southern Blot”, kedua-dua *OpMyb* telah disahkan wujud sebagai gen keluarga kecil. Ekspresi terlampau atau merosot bagi sesuatu gen kerap diperolehi daripada produksi tumbuhan ubahsuai yang mengandungi gen “sense” dan “antisense”. Oleh itu, gen *OpMyb24* yang berorientasi “sense” dan “antisense” telah berjaya dimasukkan di antara promotor “35S CaMV” dan penamat “nos” pada vektor ekspresi “p35S/NOS”. Kedua-dua jenis orientasi gen disubklonkan pada vektor binari (pCAMBIA 1301) untuk digunakan dalam pengubahsuaian kelapa sawit pada masa depan. Ini adalah salah satu pendekatan yang mungkin dapat memberi matlumat penting berkenaan fungsi gen *OpMyb24* melalui kajian fenotip kelapa sawit yang telah diubahsuaikan.

ACKNOWLEDGMENTS

The work presented in this Thesis could not have been possible without the guidance and support of many people.

In first place, I would like to thank my supervisor, Dr. Tan Siang Hee, for giving me the opportunity of pursue this work and directing my efforts to complete it. He has been an invaluable source of support and guidance all through my postgraduate program.

My thanks to Prof. Madya Dr. K. Harikrishna and Dr. Cheah Suan Choo of MPOB for agreeing to serve on my committee and taking a keen interest in my research. I am also grateful to Dr. Cheah Suan Choo for the financial support for this project.

My heartfelt thanks to Dr. Ho Chai Ling for her timely input and invaluable help in the experiments. I would also like to thank Dr. Jenni and Dr. Faridah for their support and valuable feedback to me during this project.

Thanks must also go to all members in the Genetic Lab of Faculty Food Science and Biotechnology, UPM. Hwang, Lee, Mei Chooi, Chin Ching, Au, Jason, Yang Peng, Peck Kuen, See, Choong, Chee How, Chuen Yi, Wai Har, Siew, Siti Suhaila, Siti Habsah, Rahsiah, Mr. Ong and Sequencing Officers who had shared many helpful hints, pointed out some pitfalls to avoid and spiced it all with true friendship.

A special thanks to the staff at the MPOB especially Dr. Meilina Ong, Dr. Sharifah, Siew Eng, Zaidah, Ayu, Kak Azizah, Kak Feshah and many others for providing the technical support, and their continuous and invaluable assistance.

Lastly, I would like to thank my family for their encouragement and support of my work.

I certify that an Examination Committee met on 31st October 2002 to conduct the final examination of Teoh Wan Chin on her Master of science thesis entitled “Isolation and Characterization of *Myb*-Related Genes from Oil Palm (*Elaeis guineensis* Jacq.)” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Ho Chai Ling, Ph.D.

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Chairman)

Tan Siang Hee, Ph.D.

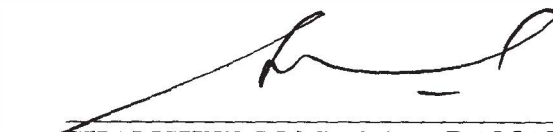
Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Harikrishna a/l Kulaveerasingam, Ph.D.

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Cheah Suan Choo, Ph.D.

Advanced Biotechnology and Breeding Centre
Research Division of Biology
Malaysian Palm Oil Board (MPOB)
(Member)



SHAMSHER MOHAMAD RAMADILI, Ph.D.,
Professor/Deputy Dean,
School of Graduate Studies,
Universiti Putra Malaysia

Date: 25 JAN 2003

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for degree of the Master of Science. The members of the Supervisory Committee are as follows:

Tan Siang Hee, Ph.D.

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Chairman)

Harikrishna a/l Kulaveerasingam, Ph.D.

Faculty of Food Science and Biotechnology
Universiti Putra Malaysia
(Member)

Cheah Suan Choo, Ph.D.

Advanced Biotechnology and Breeding Centre
Research Division of Biology
Malaysian Palm Oil Board (MPOB)
(Member)



AINI IDERIS, Ph.D.

Professor
Dean, School of Graduate Studies
Universiti Putra Malaysia

Date: **13** MAR 2003

DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

TEOH WAN CHIN

Date:

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL SHEETS	x
DECLARATION FORM	xii
TABLE OF CONTENTS	xiii
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
ABBREVIATIONS	xxiii
 CHAPTER	
 I INTRODUCTION	 1
 II LITERATURE REVIEW	
Oil Palm	
Introduction	4
Molecular studies	5
Transcription Factor	
Plants Transcription Factor	7
Transcriptional Regulators	8
Overexpression Technology	9
Antisense Technology	9
Inducible Gene Expression	10
MYB Transcription Factor	
Introduction	10
<i>MYB</i> Activity in Plants	11
Functions of Different MYB Proteins	13
Phenylpropanoid metabolism	13
Cell Shape	18
Response to Light	19
Response to Hormones	20
 III MATERIALS AND METHODS	
Plant Materials	24
cDNA Libraries	24
PCR Cloning of <i>myb</i> -related cDNA	
Genomic DNA Extraction	24
PCR Amplification	25
Purification of band of Interest from Agarose Gel	26
Cloning of PCR Product	26
Analysis of Cloned PCR Fragments	
Miniprep Plasmid Isolation	27

	Purification of Plasmid DNA by Precipitation with Polyethylene Glycol	28
	Screening of Plasmid with Insert	28
	DNA Sequencing and Computer Analysis	28
	Sample Preparation	29
	Preparation of <i>E. coli</i> XL1-Blue MRF'	
	Bacterial Culture for Infection	29
	Plating and Titering	30
	Screening of cDNA Libraries	
	Plaque Lift	31
	³² P dCTP Radiolabelling of cDNA Probe	32
	Plaque Hybridization	33
	Washing	33
	Plaque Corring	34
	<i>in vivo</i> Excision	34
	Northern Blot Analysis	
	Treatments of Plant Tissues	35
	Total RNA Extraction	36
	Total RNA Extraction from Roots and Mesocarp	37
	Northern Blotting	38
	Northern Hybridization	40
	Semi-Quantitative RT-PCR Analysis	41
	First Strand cDNA Synthesis	41
	Quantitation of Synthesized cDNA	41
	PCR Analysis with Gene Specific Primers	42
	Confirmation of the Specificity of PCR Products	43
	Genomic Southern Analysis	
	Southern Blotting	44
	Southern Hybridization	44
	Subcloning of <i>OpMyb24</i> into an Binary Vector pCAMBIA 1301	44
IV	RESULTS AND DISCUSSION	
	PCR Cloning of Oil Palm <i>Myb</i> -Related cDNA	46
	Preparation of Homologous Probe	48
	Screening of Full-Length <i>Myb</i> -Related Genes from Oil Palm	50
	Nucleotide and Deduced Amino Acid Sequences of Full Length <i>OpMyb24</i> and Partial Gene of <i>OpMyb15</i>	55
	Expression Analysis of <i>OpMyb24</i> and <i>OpMyb15</i> in Various Tissues	62
	A. <i>OpMyb15</i>	62
	B. <i>OpMyb24</i>	64
	Expression Analysis of <i>OpMyb24</i> and <i>OpMyb15</i> in Response to Gibberellic Acid Treatment	67
	Expression Analysis of <i>OpMyb24</i> in Response to Wounding	71
	Expression Analysis of <i>OpMyb24</i> in Response to HgCl ₂ Treatment	73

Expression Analysis of <i>OpMyb24</i> in Response to UV Treatment	75
Genomic Southern Analysis	77
Subcloning of <i>OpMyb24</i> into p35S/NOS Expression Vector	79
Subcloning of <i>OpMyb24</i> Gene Expression Cassette into pCAMBIA 1301 Binary Vector	82
V CONCLUSION	83
BIBLIOGRAPHY	86
APPENDIX A The Chemical and Media Formulations	101
APPENDIX B Mathematical Formula	106
APPENDIX C The Circular Map and Polylinker Sequence of the pBluescript® SK (+ / -) Phagemid	107
APPENDIX D The Circular Map of the p35S/NOS Expression Vector	108
APPENDIX E The Circular Map and Polylinker Sequence of the pCAMBIA 1301	109
BIOBATA OF AUTHOR	110

LIST OF TABLES

Table	Page
4.1 Sequences analysis of potential cDNA clones according to BLASTX results	55

LIST OF FIGURES

Figures		Page
2.1	The highly conserved N-terminal repeats of the Myb proteins were shown to function as a sequence-specific DNA-binding domain (Klempnauer and Sippel, 1987 and Biedenkapp <i>et al.</i> , 1988). The solution structure of the second and third repeats of the murine c-Myb DNA binding domain (blue) complexes to DNA (yellow and red) was solved by NMR (Ogata <i>et al.</i> , 1994). The two Myb repeats form variant helix-turn-helix structures, which lie head to tail in the major groove of the DNA (Lipsick, 1996). Adapted from http://www.stanford.edu/group/lipsick/mybstruc.htm	12
2.2	Biosynthetic pathway for Anthocyanin and Phlobaphene pigments in maize. The <i>C2</i> , <i>Chi</i> , and <i>A1</i> genes encode enzymes for chalcone synthase, chalcone isomerase, and dihydroflavonol reductase, respectively. The <i>A2</i> (Anthocyaninless2), <i>Bz1</i> (Bronze1), and <i>Bz2</i> (Bronze2) genes are required for biosynthesis of anthocyanins but not of phlobaphenes Adapted from Chopra <i>et al.</i> , 1996.....	16
2.3	A partial diagram of the phenylpropanoid pathway showing intermediates and enzymes involved in isoflavone synthesis, as well as some branch pathways. The enzymes, chalcone reductase and isoflavone synthase, are encoded by genes expressed as transgene in the study of Production of the Isoflavones Genistein and Daidzein in Non-Legume Dicot and Monocot Tissues. Genes encoding the underlined enzymes have been shown to be activated in maize by C1 and R. Dotted arrows represent multiple steps. Enzymes are indicated in italics. Adapted from Yu <i>et al.</i> , 2000.....	17
2.4	A simplified pathway for the synthesis of the sinapic ester and flavonoids derived from phenylalanine (Harborne, 1980) is presented with the relevant intermediates and products diagrammed. Circled numbers indicate enzymes: (1) PAL (EC 4.3.1.5), (2) CHS, encoded by the <i>TT4</i> gene, and (3) CHI (EC 5.5.1.6), encoded by the <i>TT5</i> gene. Adapted from Li <i>et al.</i> , 1993.....	21
2.5	Regulation of GA-inducible promoter by transacting factors. Binding of GAB1 to the α -amylase GARE represses expression of α -amylase genes in the absence of GA. GA reduces this expression by stimulating the production of the transactivator GAMYB. Binding of GAB1 to the GARE may be enhanced by	

	VPl. Adapted from Bethke <i>et al.</i> , 1997.....	23
4.1	Alignment of the deduced partial oil palm Mybs (Op6 and Op16) and other Mybs amino acid sequences, namely, MYB4 (accession NP_195574), TMH27 (accession S69189), GHMYB9 (accession AAK19619) and MYB308 (accession JQ0960). The third tryptophan residue presents in the repeat II is labelled with asterisk (*). The first tryptophan residue presents in the repeat III of plant MYB proteins replaced by phenylalanine residue is marked with arrowhead ()	47
4.2	The homologous primers, OPS and OP6AS, were designed based on the partial nucleotide sequence of <i>Op6</i>	48
4.3	The homologous primers, OPS and OP16AS, were designed based on the partial nucleotide sequence of <i>Op16</i>	49
4.4	<i>Myb-related</i> cDNA fragment in vector pBluescript SK ⁺ . Lane 1: 100 bp Plus DNA ladder (Fermentas); Lane 2: PCR amplification of pBluescript SK ⁺ using primers OPS and OP6AS; Lane 3: PCR amplification of pBluescript SK ⁺ using primers OPS and OP16AS; Lane 4: amplified DNA fragment of OpMyb6 using primers OPS and OP6AS; Lane 5: amplified DNA fragment of OpMyb16 using primers OPS and OP16AS	50
4.5	Autoradiograph of primary screening of (A) oil palm zygotic embryo cDNA library and (B) oil palm suspension culture cDNA library. Arrows show one of the putative clones was selected for secondary screening. Three darker dots indicate orientation for the membrane	51
4.6	Autoradiographs of secondary screening of (A) oil palm zygotic embryo cDNA library and (B) oil palm suspension culture cDNA library. Arrows show one of the putative clones was selected for <i>in vivo</i> excision	52
4.7	Amplification of cDNA inserts of putative clones with T3 and T7 primers. Gel electrophoresis on 1.2% (w/v) agarose gel with 1kb DNA marker in lane 1 (A) and lane 2 (B). (A) Clone 2 (lane 3), Clone 3 (lane 4), Clone 12 (lane 13), Clone 15 (lane 16), (B) Clone 19 (lane 4), Clone 24 (lane 9), Clone 25 (lane 10), Clone 26 (lane 11) and Clone 31 (lane 16) were sequenced.	53
4.8	Amplification of cDNA inserts of putative clones with T3 and T7 Primers. Gel electrophoresis on 1.2% (w/v) agarose gel with 1kb DNA marker (lane 2). Lane 1: clone 33 and lane 3 to lane 25: clone 34 to clone 56.....	54

Figures	Page
4.9 The complete nucleotide sequence and deduced amino-acid sequence of <i>OpMyb24</i> cDNA. The codons for initiation and termination of translation are in bold and marked with asterisk (*)	56
4.10 The partial nucleotide sequence and deduced amino-acid sequence of <i>OpMyb15</i> cDNA. The codons for termination of translation are in bold and marked with asterisk (*)	57
4.11 Comparison of the deduced amino acid sequences of oil palm Mybs (<i>OpMyb15</i> and <i>OpMyb24</i>) and other Myb-related proteins, namely, Zm38, C1, P and Zm1 (<i>Zea mays</i> , 227033, P10290, S26150 and 227032, respectively), MIXTA, MYB305 and MYB340 (<i>Antirrhinum majus</i> , S45338, JQ0958 and JQ0959, respectively), ATMYB4 and GL1 (<i>Arabidopsis thaliana</i> , NP 198018 and AAL01244, respectively), GAMyb (<i>Hordeum vulgare</i> , X87690). Gaps (dashed lines were inserted to optimize alignment. Sequence identity is boxed and shaded with colour. Asterisks (*) represent conserved tryptophan residues. The first tryptophan residue presents in the repeat III of plant MYB proteins replaced by another hydrophobic (phenylalanine, isoleucine, or leucine) residue is marked with arrowhead ()	59
4.12 Alignment of amino acid sequences of <i>myb</i> -related proteins. The dendrogram was constructed following multiple sequence alignment of the two repeats of reported plant Myb proteins using GENETYX-MAC 8.0. The MYB sequences used in the analysis are the following: <i>Antirrhinum majus</i> (Myb340, accession JQ0959; Myb350, accession JQ0958; Myb308, accession JQ0960; Myb330, accession JQ0957; MIXTA, accession S45338), <i>Hordeum vulgare</i> (HvGAMyb, accession X87690), <i>Perilla frutescens</i> (MYB-P1, accession BAA75512), <i>Zea mays</i> (C1, accession P10290; P, accession P27898; Zm38, accession P20025; Zm1, accession P20024), <i>Arabidopsis thaliana</i> (AtMyb2, accession BAA03534; AtMyb4, accession NP_198018; WER, accession NP_196979; GL1, accession AAL01244), <i>Nicotiana tabacum</i> (NtMyb1, accession T03850), <i>Oryza sativa</i> (OsGAMyb, accession CAA67000; Cpm7, Accession BAB67851; OsJAMyb, accession AAK08983), <i>Lolium temulentum</i> (LtGAMyb, accession AAD31395), <i>Craterostigma plantagineum</i> (Cpm10, accession T09735).....	61
4.13 Semi-quantitative RT-PCR analysis. Twenty-five nanogram of the cDNA was used in PCR with <i>OpMyb15</i> gene specific primers. Three microlitre of each sample was removed at 25, 30, 35, 40 and 45 cycles, and 0.5 µl of each sample was used for ethidium	

- bromide plate assay. Lane1: control without cDNA; lane 2: 28-cm female flowers; lane 3: 28-cm male flowers; lane 4: 1-2-cm female flowers; lane 5: 1-2-cm male flowers; lane 6: roots; lane 7: mesocarps; lane 8: three-month-old leaves; lane 9: meristems; lane 10: young leaves..... 63
- 4.14 Expression level of *OpMyb15* in different tissues and (B) confirmation of specificity of semi-quantitative RT-PCR analysis. Lane1: control without cDNA; lane 2: 28-cm female flowers; lane 3: 28-cm male flowers; lane 4: 1-2-cm female flowers; lane 5: 1-2-cm male flowers; lane 6: roots; lane 7: mesocarps; lane 8: three-month-old leaves; lane 9: meristems; lane 10: young leaves; lane11: 1kb DNA marker..... 64
- 4.15 Semi-quantitative RT-PCR analysis. Twenty-five nanogram of the cDNA was used in PCR with *OpMyb24* gene specific primers. Three microliter of each samples was aliquated at 25, 30, 35, 40 and 45 cycles, and 0.5 µl of each samples was used for ethidium bromide plate assay. Lane1: control without cDNA; lane 2: 28-cm female flowers; lane 3: 28-cm male flowers; lane 4: 1-2-cm female flowers; lane 5: 1-2-cm male flowers; lane 6: roots; lane 7: mesocarps; lane 8: three-month-old leaves; lane 9: meristems; lane 10: young leaves..... 65
- 4.16 (A) Expression level of *OpMyb24* in different tissues and (B) confirmation of specificity of semi-quantitative RT-PCR analysis. Lane1: control without cDNA; lane 2: 28-cm female flowers; lane 3: 28-cm male flowers; lane 4: 1-2-cm female flowers; lane 5: 1-2-cm male flowers; lane 6: roots; lane 7: mesocarps; lane 8: three-month-old leaves; lane 9: meristems; lane 10: young leaves; lane 11: 1kb DNA marker 65
- 4.17 Semi-quantitative RT-PCR analysis. Twenty-five nanogram of the cDNA was used in PCR with *OpMyb15* gene specific primers and 0.5 µl of each sample was used for ethidium bromide plate assay. *In vitro* seedlings were incubated for 0 hr (lane1), 3 hr (lane 2), 6 hr (lane 3), 12 hr (lane 4), 18 hr (lane 5), 24 hr (lane 6), 48 hr (lane 7) in culture medium with (100 µM) or without (0 µM) GA₃. 68
- 4.18 Expression level of *OpMyb15* in (A) untreated and (B) *in vitro* seedlings treated with 100 µM GA₃. Each lane indicates hours after exposure to treatment. Lane1: 0 hr; lane 2: 3 hr; lane 3: 6 hr; lane 4: 12 hr; lane 5: 18 hr; lane 6: 24 hr; lane 7: 48 hr; lane 8: control without cDNA; lane 9: 1kb DNA marker 68

4.19	Confirmation of specificity of semi-quantitative RT-PCR analysis of <i>OpMyb15</i> in (A) untreated and (B) <i>in vitro</i> seedlings treated with 100 μ M GA ₃ after 0 hr (lane 1), 3 hr (lane 2), 6 hr (lane 3), 12 hr (lane 4), 18 hr (lane 5), 24 hr (lane 6), 48 hr (lane 7), control without cDNA (lane 8).....	69
4.20	Semi-quantitative RT-PCR analysis. Twenty-five nanogram of the cDNA was used in PCR with <i>OpMyb24</i> gene specific primers and 0.5 μ l of each samples was used for ethidium bromide plate assay. <i>In vitro</i> seedlings were incubated for 0 hr (lane 1), 3 hr (lane 2), 6 hr (lane 3), 12 hr (lane 4), 18 hr (lane 5), 24 hr (lane 6), 48 hr (lane 7) in culture medium with (100 μ M) or without (0 μ M) GA ₃	69
4.21	Expression level of <i>OpMyb24</i> in (A) untreated and (B) <i>in vitro</i> seedlings treated with 100 μ M GA ₃ . Each lane indicates hours after the start of the treatment. Lane1: 0 hr; lane 2: 3 hr; lane 3: 6 hr; lane 4: 12 hr; lane 5: 18 hr; lane 6: 24 hr; lane 7: 48 hr; lane 8: control without cDNA; lane 9: 1kb DNA marker.....	70
4.22	Confirmation of specificity of semi-quantitative RT-PCR analysis of <i>OpMyb24</i> in (A) untreated and (B) <i>in vitro</i> seedlings treated with 100 μ M GA ₃ after 0 hr (lane 1), 3 hr (lane 2), 6 hr (lane 3), 12 hr (lane 4), 18 hr (lane 5), 24 hr (lane 6), 48 hr (lane 7), control without cDNA (lane 8).....	70
4.23	Northern analysis of <i>OpMyb24</i> gene in one-month-old oil palm <i>in vitro</i> seedlings for different periods after wounding. Autoradiographs of a RNA blot containing about 20 μ g of total RNA isolated from non-wounded (Lane 1) or wounded <i>in vitro</i> seedlings for 2 hr (lane 2), 4 hr (lane 3), 6 hr (lane 4), h and 12 hr (lane 5) hybridized with the radiolabeled <i>OpMyb24</i> cDNA fragment and re-probed with cyclophilin (<i>CyP</i>) as a loading control.....	72
4.24	Northern analysis of <i>OpMyb24</i> in one-month-old oil palm <i>in vitro</i> seedlings for different periods after HgCl ₂ treatment. Autoradiographs of a RNA blot containing about 20 μ g of total RNA isolated from non-treated (0 hr) (lane 1) or treated <i>in vitro</i> seedlings with HgCl ₂ for ½ hr (lane 2), 1 hr (lane 3) and 2 hr (lane 4), hybridized with the radiolabeled <i>OpMyb24</i> cDNA fragment and re-probed with cyclophilin (<i>CyP</i>) as a loading control	74

- 4.25 Northern analysis of *OpMyb24* in one-month-old oil palm *in vitro* seedlings for different periods after UV light exposure. Autoradiographs of a RNA blot from *in vitro* seedlings exposed with UV light (1200 μ Joules x 100) for 10 min (lane 2), 15 min (lane 3), 20 min (lane 4) and 25 min (lane 5), hybridized with the radiolabeled (A) *OpMyb24* cDNA fragment and re-probed with (B) cyclophilin (*CyP*) as a loading control. Lane 1 represents the total RNA isolated from non-treated *in vitro* seedlings and used as control in this experiment76
- 4.26 DNA Gel Blot Analysis of the *OpMyb* Genes in Oil Palm Genomic DNA. *EcoRI* was used to digest 20 μ g (lane 2), 40 μ g (lane 3) and 60 μ g (lane 4) genomic DNA; *BamHI* was used to digest 20 μ g (lane 5), 40 μ g (lane 6) and 60 μ g (lane 7) genomic DNA; *KpnI* was used to digest 20 μ g (lane 8), 40 μ g (lane 9) and 60 μ g (lane 10) genomic DNA. The blot was hybridized with (A) *OpMyb15* cDNA probe or (B) a full-length *OpMyb24* cDNA probe 78
- 4.27 The cDNA sequence that encodes for *OpMyb24*. The sequence in bold on top showed the start codon, whereas the sequence in bold below showed the stop codons. The sequences underlined indicate the restriction site for *SpeI* 80
- 4.28 Restriction enzyme analysis of p35S/NOS-Mybs [1.5% (w/v) agarose gel]. Clones p35S/NOS-Mybs were digested with *KpnI* and *SacI*. Sense constructs showed DNA fragment of 0.95 kb, while antisense constructs were digested into two DNA fragments consisting of 0.53 kb and 1.25 kb. Lane 1: Clone p35S/NOS-Myb8AS; lane 2: Clone p35S/NOS-Myb10AS; lane 3: Clone p35S/NOS-Myb12S; lane 4: Clone p35S/NOS-Myb20S; lane 5: Clone p35S/NOS-Myb22S; lane 6: Clone p35S/NOS-Myb23AS and lane 7: 1 kb DNA marker..... 82

ABBREVIATIONS

μg	-	microgram
μl	-	microliter
2-BE	-	ethylene glycol monobutyl ether
BLAST	-	Basic Local Alignment Research Tool
bp	-	base pair
cDNA	-	complementary DNA
CsCl	-	Cesium chloride
CTAB	-	Hexadecyltrimethylammonium bromide
DEPC	-	diethyl pyrocarbonate
dH ₂ O	-	distilled water
DNA	-	deoxyribonucleic acid
dNTPs	-	deoxynucleotide triphosphate
DTT	-	dithiothreitol
EDTA	-	ethylenediamine tetraacetic acid
EGTA	-	ethylene glycol-bis (β-aminoethylether)- <i>N,N,N',N'</i> -
GSH	-	glutathione
GTE	-	Glucose-Tris-EDTA
GUS	-	β-glucononidase
HCl	-	hydrochloride acid
Jacq.	-	Jacquin
kb	-	kilobase pair
KCl	-	potassium chloride
LB	-	Luria-Bertani
LiCl	-	lithium chloride
M	-	Molar

MgCl ₂	-	magnesium chloride
MgSO ₄ .7H ₂ O	-	magnesium sulfate heptahydrat
Min	-	minute(s)
Mm	-	millimolar
MOPS	-	3-(N-morpholino) propanesulfonic acid
mRNA	-	messenger RNA
MYB	-	myblastosis
N	-	Normal
NaCl	-	sodium chloride
NaOH	-	sodium hydroxide
NCBI	-	National; Center for Biotechnology Information
ng	-	nanogram
NMR	-	Nuclear Magnetic Resonance
OD _{600nm}	-	Optical density at 600 nanometer
Oligo(dT)	-	oligodeoxythymidylic acid
ORF	-	open reading frame
PAL	-	phenylalanine ammonia-lyase
PCI	-	phenol-chloroform-isoamyl alcohol
PCR	-	polymerase chain reaction
pfu	-	plaque-forming units
Poly(A) ⁺	-	polyadenylated (mRNA)
PVPP	-	polyvinylpolypyrrolidone
QTL	-	Quantitative Trait Locus
RNA	-	ribonucleic acid
RNase	-	ribonuclease
Rpm	-	revolution per minute
RT	-	reverse transcriptase